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# Peritoneal dialysis solutions reverse the hemodynamic effects of nitric oxide synthesis inhibitors

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**Peritoneal dialysis solutions reverse the hemodynamic effects of nitric oxide synthesis inhibitors.** Nitric oxide (NO) synthesis is inhibited by a variety of L-arginine analogs including $N^G$-nitro-L-arginine methyl ester (L-NAME) and $N^G$ $N^G$-dimethylarginine (ADMA). ADMA is present in elevated concentrations in renal failure and potentially could alter microcirculatory hemodynamics during peritoneal dialysis (PD). This investigation utilized the techniques of intravital microscopy to quantitate the mesenteric arteriolar hemodynamic effects of PD solutions during NO synthesis inhibition. L-NAME (100 $\mu$M) produced maximum arteriolar vasoconstriction to 74% of baseline diameter (19.9 ± 2.2 vs. 26.9 ± 1.4 $\mu$m, $P < 0.001$, $N = 10$) and ADMA (100 $\mu$M) to 68% (20.5 ± 2.5 vs. 30.1 ± 2.0 $\mu$m, $P < 0.01$, $N = 6$). L-NAME decreased red blood cell velocity to 44% of baseline velocity (3.8 ± 0.8 vs. 8.5 ± 1.1 mm/second, $P < 0.001$) and ADMA to 52% (5.1 ± 1.1 vs. 9.8 ± 0.9 mm/second, $P < 0.01$, $N = 6$). Despite NO synthesis inhibition, standard PD solutions reversed these hemodynamic effects with both 1.5% and 4.25% Dianeal (Baxter) rapidly reversing the vasoconstriction and restoring blood flow back to baseline values. When Dianeal and L-NAME were simultaneously superfused, no L-NAME induced vasoconstriction occurred and Dianeal maintained vasodilatory properties despite L-NAME ($P < 0.01$, $N = 5$). This investigation reaffirms that basal levels of NO are important in maintaining normal hemodynamics in the mesenteric microcirculation. Reversal of the L-NAME induced arteriolar hemodynamic effects by Dianeal suggests that the endogenous NO synthesis inhibitor ADMA has no significant effects in the regulation of the mesenteric microvascular arteriolar hemodynamics during PD. Since these PD solutions remain vasoactive despite NO synthesis inhibition, this suggests that these PD solutions possess vasoactive properties primarily through a NO independent mechanism.

Nitric oxide is increasingly recognized as an important bioregulatory molecule of the microcirculation affecting numerous fundamental microcirculatory processes. Inhibition of nitric oxide synthesis results in significant renal and mesenteric arteriolar vasoconstriction, increases *in vivo* microvascular permeability and acts as a pro-inflammatory stimulus (as evidenced by a marked increase in the number of adherent leukocytes in postcapillary venules), [1–4]. Nitric oxide is synthesized from L-arginine by a family of enzymes known as nitric oxide synthases (NOS), [5]. NOS is present in two major forms: a constitutive form and an inducible form [6]. The constitutive form of NOS present in endothelial cells may be inhibited by a variety of arginine analogs, including the exogenous arginine derivative $N^G$-nitro-L-arginine methyl ester (L-NAME) and the endogenous derivative $N^G$ $N^G$-dimethylarginine (also known as asymmetrical dimethylarginine, ADMA) [1, 2, 7]. Recently, ADMA has been reported to be present in elevated concentrations in chronic renal failure [8]. Patients with end-stage renal disease (ESRD) may have plasma concentrations of ADMA in the 5 $\mu$M range, which is approximately eight fold greater than patients with normal renal function [8–10]. This observation could be particularly significant for patients with ESRD who are maintained on chronic peritoneal dialysis since the accumulation of ADMA may have important consequences in regulation of the mesenteric microvascular hemodynamics during peritoneal dialysis.

Peritoneal dialysis solutions have been previously shown to possess vasoactive properties in the mesenteric microcirculation [11, 12]. Superfusion of a hypertonic peritoneal dialysis solution on arterioles produces a transient vasoconstriction followed by a sustained vasodilation. The vasoactive properties of these solutions have been attributed to the solution's hyperosmolarity and acetate or lactate buffers [13]. Recently, it has been demonstrated that hyperosmolar sodium solutions perfused into intestinal lymph produce vasodilation of submucosal intestinal arterioles through a mechanism partly mediated by endothelium-derived relaxing factor (nitric oxide), [14]. Whether or not hyperosmolar peritoneal dialysis solutions produce arteriolar vasodilation through a nitric oxide dependent mechanism, and how nitric oxide inhibitors affect the vasoactive properties of peritoneal dialysis solutions has been previously unknown. If hypertonic peritoneal dialysis solutions produce vasodilation through a nitric oxide dependent mechanism then elevated concentrations of endogenous inhibitors of nitric oxide synthesis as seen in ESRD potentially could alter the vasoactive properties of standard peritoneal dialysis solutions. In order to investigate these possibilities *in vivo* hemodynamic studies of mesenteric arterioles were performed using a standard peritoneal dialysis solution (Dianeal; Baxter Health Care Corporation, Deerfield, IL, USA) during conditions of nitric oxide synthesis inhibition.

## Methods

Male Sprague-Dawley rats (125 to 350 g) were anesthetized with Inactin (thiobutabarbital sodium) 120 mg/kg intraperitoneally. A tracheotomy was performed to maintain a patent airway and the right carotid artery was canulated for blood pressure measurement. The rat was placed in the supine position on a plexiglass microscopic stage and a midline abdominal incision was made. A segment of the small bowel was exposed through the

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midline incision and the mesentery was prepared for *in vivo* microscopic observation. The mesentery was draped over a glass cover slip approximately 2 $cm^2$ and the exposed bowel was covered with moist gauze to prevent tissue dehydration [15]. The rat was maintained at physiologic temperature using a warming lamp. A mesenteric arteriole (20 to 35 $\mu$m) was identified for study using an inverted microscope (Fluovert FS, Leitz, Germany). The mesentery was continuously superfused with a nonvasoactive peritoneal dialysis solution (NVAPD) at 3 ml/min followed by the appropriate experimental solution as outlined in the experimental protocols below. The composition of the NVAPD solution was sodium 140 mEq/liter, calcium 3.5 mEq/liter, potassium 3 mEq/liter, magnesium 2.5 mEq/liter, chloride 126 mEq/liter, bicarbonate 20 mEq/liter, sulfate 2.5 mEq/liter and dextrose 120 mg/dl (modified from [16]). The composition of 1.5% Dianeal was sodium 132 mEq/liter, calcium 3.5 mEq/liter, magnesium 0.5 mEq/liter, chloride 96 mEq/liter, lactate 40 mEq/liter and dextrose 1500 mg/dl, with a calculated osmolality of 346 mOsm/liter. The composition of 4.25% Dianeal was sodium 132 mEq/liter, calcium 3.5 mEq/liter, magnesium 0.5 mEq/liter, chloride 96 mEq/liter, lactate 40 mEq/liter and dextrose 4250 mg/dl, with a calculated osmolality of 485 mOsm/liter. The experimental solutions were bubbled with a mixture of 5%$CO_2$ and 95%$N_2$ prior to superfusion.

A video camera (Dage-MTI CCD, C72, Michigan City, IN, USA) mounted on the microscope projected the image onto a video monitor (SONY1943MD, SONY, Tokyo, Japan) and the images were recorded with a videocassette recorder (SONY 9500MD). A video time-date generator (Panasonic WJ-810, Japan) projected the time, date and stopwatch function onto the monitor. The blood pressure was monitored with a pressure transducer (1050 BPR, Biopac Systems Inc., Goleta, CA, USA) connected to a DA 100 amplifier (Biopac Systems Inc.) and an analog to digital converter (MP100, Biopac Systems Inc.). The temperature was monitored with a temperature probe (TSD 102A, Biopac Systems Inc.) connected to a SKT100 amplifier (Biopac Systems Inc.) and an analog to digital converter MP100. Arteriole microvascular diameter was measured on line using a videocaliper (Microcirculation Research Institute, Texas A&M University, College Station, TX, USA). Center line red blood cell velocity ($V_{RBC}$) was measured using an optical Doppler velocimeter (Microcirculation Research Institute). Both the videocaliper and the velocimeter were connected to a Universal Interface Module UIM 100 (Biopac Systems Inc.) and an MP100 analog to digital converter. Arteriole diameter, $V_{RBC}$, blood pressure and temperature were continuously monitored with the information downloaded to a Macintosh Quadra 650 computer (Apple) using the Acknowledge 3.0 program (Biopac Systems Inc.). The videocaliper was calibrated using a slide objective micrometer and the doppler velocimeter was calibrated using a rotating disk coated with red blood cells.

### *Experimental protocols*

Single, unbranched mesenteric arterioles (20 to 35 $\mu$m) were identified for study. The study consisted of five experimental protocols: a Control Group and Experimental Groups 1 to 4. Figure 1 outlines the different solution time periods and illustrates the transition between experimental solutions in each group. All experimental solutions were warmed to 37°C prior to mesenteric superfusion and superfused at 3 ml/min.

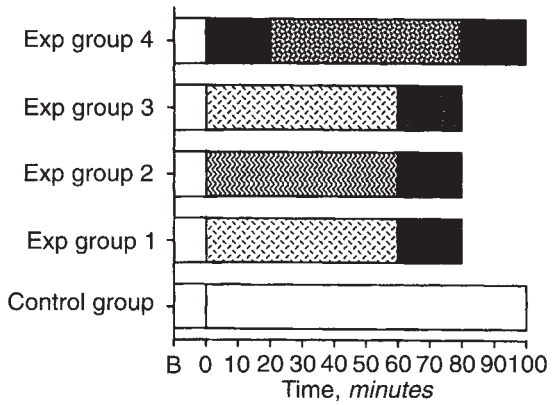



**Fig. 1.** *An experimental time line for the superfused solutions for each Experimental Group.* Abbreviations are: B, baseline period; NVAPD, nonvasoactive peritoneal dialysis solution. Symbols are: (□) NVAPD; (▨) NVAPD + L-NAME; (▧) NVAPD + ADMA; (■) 4.25% Dianeal; (▮) 1.5% Dianeal; (▬) 4.25 Dianeal + L-NAME.

*Control Group.* The stability of the experimental preparation over time was tested by superfusing the mesentery with the NVAPD control solution. As in each experimental group, arteriole vessel diameter, center line red blood cell velocity ($V_{RBC}$) and blood pressure were continuously monitored and recorded. This information was then available for future data analysis.

*Experimental Groups 1, 2 and 3.* The objective of these Experimental Groups was to determine the arteriole hemodynamic effects of the inhibitors of nitric oxide synthesis, L-NAME and ADMA, and to examine the hemodynamic effects of standard hyperosmolar peritoneal dialysis solutions (1.5% or 4.25% Dianeal) following nitric oxide synthesis inhibition. The mesentery was superfused for 40 minutes with NVAPD solution (30-min stabilization period followed by a 10-min baseline period). Following the baseline period, 100 $\mu$M L-NAME or ADMA was added to the NVAPD solution and superfused for 60 minutes. The superfused solution was then changed to 4.25% Dianeal for Experimental Groups 1 and 2 or 1.5% Dianeal for Experimental Group 3 and superfused for 20 minutes.

*Experimental Group 4.* The objective of Experimental Group 4 was to determine the arteriole hemodynamic effects of simultaneous superfusion of L-NAME and 4.25% Dianeal. The mesentery was superfused for 40 minutes with NVAPD solution (30-min stabilization period followed by a 10-min baseline period). Following the baseline period, 4.25% Dianeal was superfused for 20 minutes. Then 100 $\mu$M L-NAME was added to the 4.25% Dianeal solution and superfused for 60 minutes. The superfused solution was then changed back to 4.25% Dianeal for 20 minutes.

### *Statistical analysis*

All values for each 10-minute time period were expressed as means ± SEM. The data were analyzed using an analysis of variance with Tukey's post-hoc test. Statistical significance was set at $P < 0.05$.

## Results

In the Control Group there were no significant differences in mean values for arteriole diameter or $V_{RBC}$ in any 10 minute time

**Table 1.** Summary of mean arteriole hemodynamic data

| Solution | NVAPD | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time *min* | Baseline | 0 to 10 | 10 to 20 | 20 to 30 | 30 to 40 | 40 to 50 | 50 to 60 | 60 to 70 | 70 to 80 | 80 to 90 | 90 to 100 |
| Control Group NVAPD | | | | | | | | | | | |
| Diameter $\mu m$ | 25.6 ± 0.7 | 25.6 ± 0.7 | 25.6 ± 0.7 | 25.6 ± 0.7 | 25.6 ± 0.7 | 25.6 ± 0.7 | 25.6 ± 0.7 | 25.6 ± 0.7 | 25.6 ± 0.7 | 25.4 ± 0.7 | 25.5 ± 0.9 |
| RBC Velocity *mm/sec* | 7.9 ± 1.3 | 8.0 ± 1.4 | 8.1 ± 1.3 | 9.2 ± 1.4 | 9.4 ± 1.5 | 9.3 ± 1.5 | 9.6 ± 1.6 | 10.4 ± 1.4 | 8.3 ± 2.0 | 8.4 ± 2.3 | 8.7 ± 1.6 |

| Solution | NVAPD | NVAPD + 100 $\mu$M L-NAME or ADMA | | | | | | 4.25 or 1.5% Dianeal | |
|---|---|---|---|---|---|---|---|---|---|
| Time *min* | Baseline | 0 to 10 | 10 to 20 | 20 to 30 | 30 to 40 | 40 to 50 | 50 to 60 | 60 to 70 | 70 to 80 |
| Experimental Group 1 L-NAME, 4.25% Dianeal | | | | | | | | | |
| Diameter $\mu m$ | 28.0 ± 2.8 | 25.0 ± 2.7 | 24.8 ± 3.6 | 22.8 ± 4.1 | 21.2 ± 4.6 | 20.2 ± 3.7 | 22.2 ± 4.1 | 29.2 ± 5.1 | 31.6 ± 3.8 |
| RBC Velocity *mm/sec* | 8.2 ±2.2 | 7.1 ± 1.6 | 5.2 ± 1.7 | 4.6 ± 1.6 | 3.9 ± 1.6 | 3.8 ± 1.6 | 3.6 ± 1.5 | 8.1 ± 2.0 | 8.3 ± 1.6 |
| Experimental Group 2 ADMA, 4.25% Dianeal | | | | | | | | | |
| Diameter $\mu m$ | 30.1 ± 2.0 | 30.1 ± 2.0 | 28.3 ± 1.7 | 26.5 ± 1.8 | 23.3 ± 2.2 | 21.5 ± 2.5 | 20.5 ± 2.5 | 30.0 ± 2.4 | 32.5 ± 2.5 |
| RBC Velocity *mm/sec* | 9.8 ± 0.9 | 8.7 ± 0.6 | 7.2 ± 0.4 | 8.4 ± 0.7 | 6.4 ± 0.6 | 5.7 ± 0.7 | 5.1 ± 1.1 | 8.9 ± 1.1 | 9.6 ± 0.9 |
| Experimental Group 3 L-NAME, 1.5% Dianeal | | | | | | | | | |
| Diameter $\mu m$ | 25.8 ± 0.7 | 25.4 ± 0.5 | 24.8 ± 0.7 | 23.4 ± 0.5 | 21.4 ± 1.4 | 20.4 ± 1.8 | 17.6 ± 1.7 | 23.4 ± 0.7 | 27.0 ± 0.7 |
| RBC Velocity *mm/sec* | 8.9 ± 0.7 | 8.1 ± 0.7 | 7.2 ± 1.2 | 6.4 ± 1.3 | 5.7 ± 1.2 | 4.2 ± 0.8 | 3.9 ± 0.5 | 7.9 ± 1.2 | 8.1 ± 1.4 |

| Solution | NVAPD | 4.25% Dianeal | | 4.25% Dianeal + 100 $\mu$M L-NAME | | | | | | 4.25% Dianeal | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Time *min* | Baseline | 0 to 10 | 10 to 20 | 20 to 30 | 30 to 40 | 40 to 50 | 50 to 60 | 60 to 70 | 70 to 80 | 80 to 90 | 90 to 100 |
| Experimental Group 4 L-NAME in 4.25% Dianeal | | | | | | | | | | | |
| Diameter $\mu m$ | 28.0 ± 2.5 | 30.6 ± 2.8 | 32.6 ± 3.2 | 32.2 ± 3.3 | 31.8 ± 3.2 | 32.2 ± 3.1 | 31.4 ± 3.4 | 31.8 ± 3.2 | 31.0 ± 3.3 | 31.6 ± 3.0 | 31.6 ± 3.2 |
| RBC velocity *mm/sec* | 8.9 ± 1.2 | 9.7 ± 1.7 | 10.9 ± 1.5 | 10.3 ± 1.5 | 9.8 ± 1.3 | 8.4 ± 1.7 | 7.4 ± 1.9 | 7.6 ± 1.9 | 6.9 ± 2.1 | 7.4 ± 1.7 | 8.1 ± 1.6 |

Abbreviation is: NVAPD, nonvasoactive peritoneal dialysis solution. Values are means ± SEM.

period. This demonstrates the stability of the experimental preparation over time. In Experimental Group 1, when compared to the baseline period, 100 $\mu$M L-NAME produced significant arteriolar vasoconstriction (maximum vasoconstriction occurring in the 40 to 50 min time interval: 20.0 ± 3.7 vs. 28.0 ± 2.8 $\mu$m, $P = 0.01$, $N = 5$) and decreased $V_{RBC}$ (maximum decline in $V_{RBC}$ occurring in the 50 to 60 min time interval: 3.6 ± 1.5 vs. 8.2 ± 2.2 mm/second, $P < 0.05$, $N = 5$). 4.25% Dianeal reversed the L-NAME induced vasoconstriction (31.6 ± 3.8 vs. 20.0 ± 3.7 $\mu$m, $P < 0.01$, $N = 5$) and restored $V_{RBC}$ (8.3 ± 1.6 vs. 3.6 ± 1.5 mm/second, $P < 0.05$, $N = 5$). In Experimental Group 2, when compared to the baseline period, 100 $\mu$M ADMA produced significant arteriolar vasoconstriction (maximum vasoconstriction occurring in the 50 to 60 min time interval: 20.5 ± 2.5 vs. 30.1 ± 2.0 $\mu$m, $P < 0.01$, $N = 6$) and decreased $V_{RBC}$ (maximum decline in $V_{RBC}$ occurring in the 50 to 60 min time interval: 5.1 ± 1.1 vs. 9.8 ± 0.9 mm/sec, $P < 0.01$, $N = 6$). The 4.25% Dianeal reversed the ADMA induced vasoconstriction (32.5 ± 2.5 vs. 20.5 ± 2.5 $\mu$m, $P < 0.001$, $N = 6$) and restored $V_{RBC}$ (9.6 ± 0.9 vs. 5.1 ± 1.1 mm/second, $P \leq 0.01$, $N = 6$). In Experimental Group 3, when compared to the baseline period, 100 $\mu$M L-NAME produced significant arteriolar vasoconstriction (maximum vasoconstriction occurring in the 50 to 60 min time interval: 17.6 ± 1.7 vs. 25.8 ± 0.7 $\mu$m, $P < 0.01$, $N = 5$) and decreased $V_{RBC}$ (maximum decline in $V_{RBC}$ occurring in the 50 to 60 min time interval: 3.9 ± 0.5 vs. 8.9 ± 0.7 mm/second, $P < 0.01$, $N = 5$). The 1.5% Dianeal reversed the L-NAME induced vasoconstriction (27.0 ± 0.7 vs. 17.6 ± 1.7 $\mu$m, $P < 0.01$, $N = 5$) and restored $V_{RBC}$ (8.1 ± 1.4 vs. 3.9 ± 0.5 mm/second, $P < 0.01$, $N = 5$). Table 1 gives the mean hemodynamic data for the Control and Experimental Groups 1 through 4. The data for arteriole diameter and $V_{RBC}$ for Experimental Groups 1 to 3 are graphically illustrated in Figures 2 to 4, respectively. Figure 5 shows a comparison in the percent change in the arteriole diameter for Experimental Groups 1 to 3, and Figure 6 shows a comparison in the percent change in the $V_{RBC}$ for Experimental Groups 1 to 3. These data also demonstrate that the significant changes in arteriole diameter and $V_{RBC}$ secondary to basal nitric oxide production inhibition occur primarily in the second 30-minute period of the 60-minute L-NAME or ADMA superfusion period. In addition, further studies demonstrated that L-NAME induced vasoconstriction was reversed with pH adjusted 4.25% Dianeal (pH = 7.38, $P < 0.01$, $N = 4$). This is in agreement with Miller et al, who found it unlikely that low pH accounted for the vasoactive properties of peritoneal dialysis solutions since adjustment of pH to 7.0 to 7.4 did not alter vasodilator responses in the rat cremaster muscle [11].

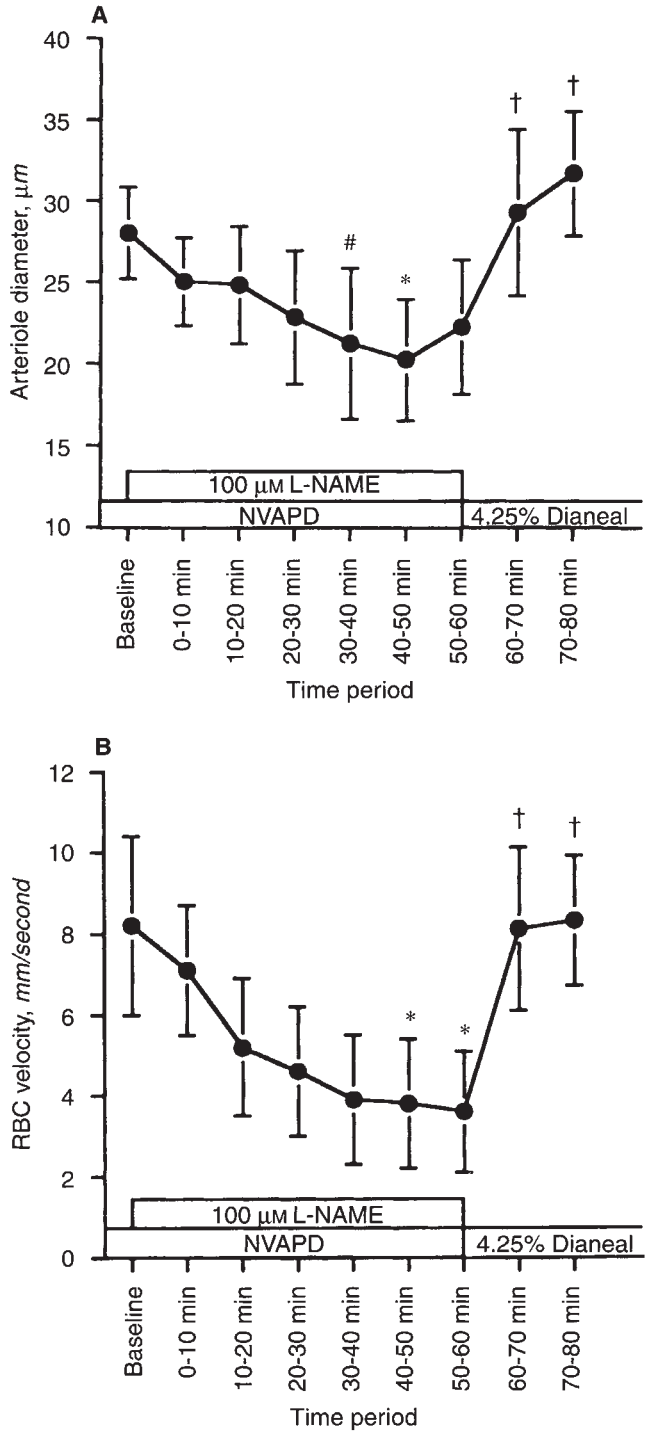



**Fig. 2.** *Hemodynamic data for Experimental Group 1.* **A.** Arteriole diameter. L-NAME induced vasoconstriction, $\#P < 0.05$ and $*P = 0.01$ when compared to the baseline period; 4.25% Dianeal reversed the L-NAME induced vasoconstriction, $\dagger P < 0.01$ when compared to the period of maximal vasoconstriction (40 to 50 min). **B.** Red blood cell velocity ($V_{RBC}$). L-NAME induced decrease in $V_{RBC}$, $*P < 0.05$ when compared to the baseline period; 4.25% Dianeal reversed the L-NAME induced decrement in $V_{RBC}$, $\dagger P < 0.05$ when compared to the period of maximal decline in $V_{RBC}$ (50 to 60 min), $N = 5$.

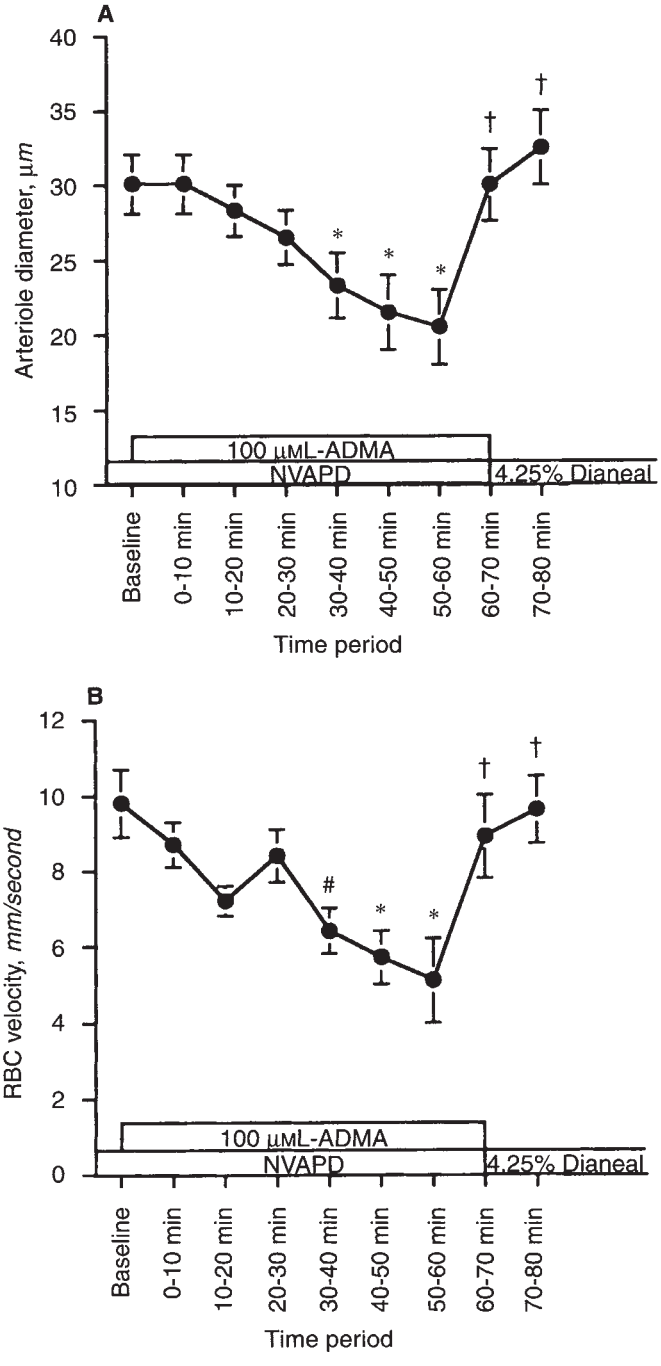
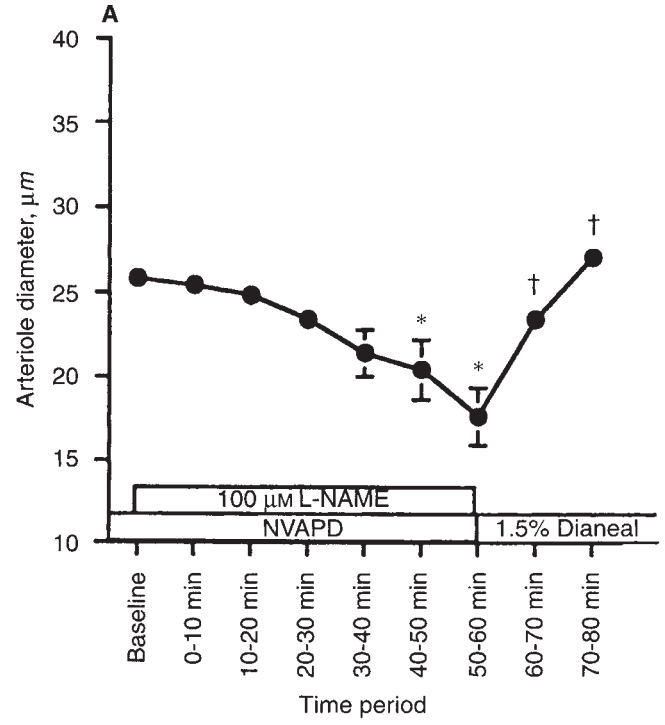



**Fig. 3.** *Hemodynamic data for Experimental Group 2.* **A.** Arteriole diameter. ADMA induced vasoconstriction, $*P < 0.01$ when compared to the baseline period; 4.25% Dianeal reversed the ADMA induced vasoconstriction, $\dagger P < 0.001$ when compared to the period of maximal vasoconstriction (50 to 60 min). **B.** Red blood cell velocity ($V_{RBC}$). ADMA induced decrease in $V_{RBC}$, $\#P < 0.05$ and $*P < 0.01$ when compared to the baseline period; 4.25% Dianeal reversed the ADMA induced decrement in $V_{RBC}$, $\dagger P \leq 0.01$ when compared to the period of maximal decline in $V_{RBC}$ (50 to 60 min), $N = 6$.

Combining the data for each time period in L-NAME from Experimental Groups 1 and 3 demonstrates that L-NAME (100 μM) produces maximum arteriolar vasoconstriction to 74% of baseline diameter (19.9 ± 2.2 vs. 26.9 ± 1.4 μm, $P < 0.001$, $N = 10$) as compared to ADMA (100 μM) to 68% of baseline diameter (20.5 ± 2.5 vs. 30.1 ± 2.0 μm, $P < 0.01$, $N = 6$). In addition, L-NAME decreases $V_{RBC}$ to 44% of baseline velocity (3.8 ± 0.8 vs. 8.5 ± 1.1 mm/second, $P < 0.001$, $N = 10$) while ADMA decreases $V_{RBC}$ to 52% of baseline (5.1 ± 1.1 vs. 9.8 ± 0.9



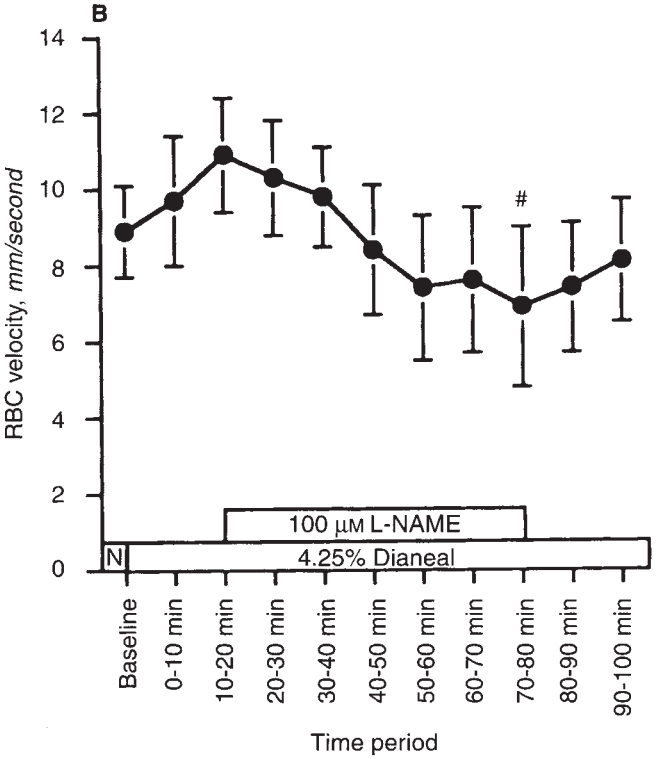




**Fig. 4.** *Hemodynamic data for Experimental Group 3.* **A.** Arteriole diameter. L-NAME induced vasoconstriction, $^*P \leq 0.01$ when compared to the baseline period; 1.5% Dianeal reversed the L-NAME induced vasoconstriction, $\dagger P < 0.01$ when compared to the period of maximal vasoconstriction (50 to 60 min). **B.** Red blood cell velocity ($V_{RBC}$). L-NAME induced decrease in $V_{RBC}$, $\#P < 0.05$ and $^*P < 0.01$ when compared to the baseline period; 1.5% Dianeal reversed the L-NAME induced decrement in $V_{RBC}$, $\dagger P < 0.01$ when compared to the period of maximal decline in $V_{RBC}$ (50 to 60 min), $N = 5$.

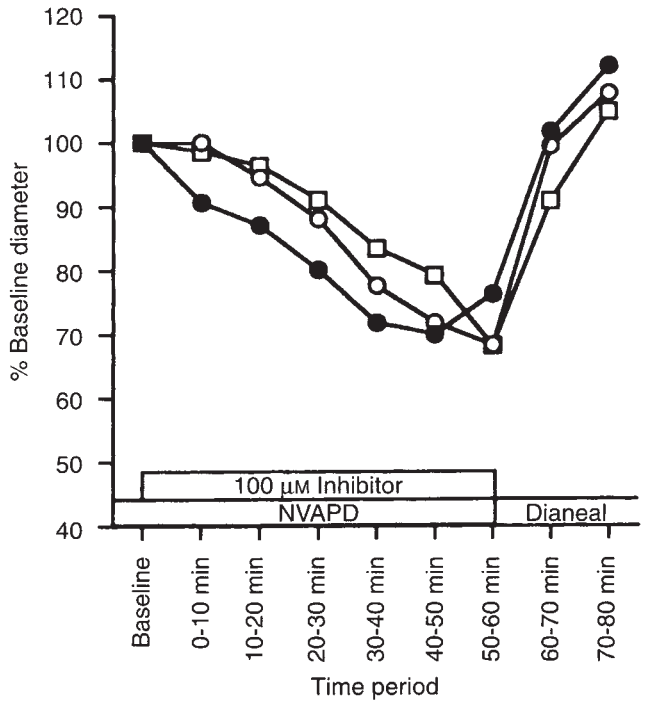



**Fig. 5.** *A comparison of the percent change in the arteriole diameter for Experimental Groups 1 to 3.* Symbols are: (●) Group 1; (○) Group 2; (□) Group 3.

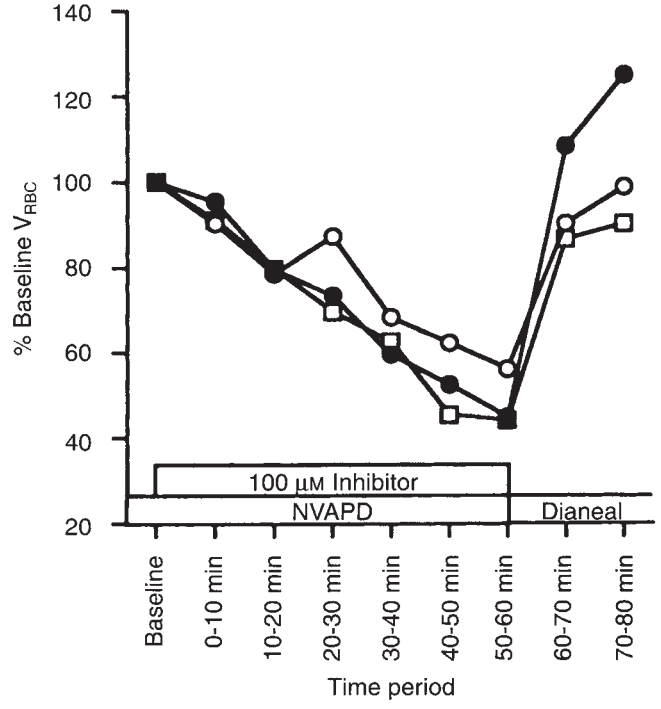



**Fig. 6.** *A comparison of the percent change in the $V_{RBC}$ for Experimental Groups 1 to 3.* Symbols are: (●) Group 1; (○) Group 2; (□) Group 3.

mm/second, $P < 0.01$, $N = 6$). Figure 7 is a photomicrograph of an intravital microscopic image of an arteriole during an experiment with ADMA and Dianeal.

In Experimental Group 4 the arteriole hemodynamic effects of simultaneous superfusion of L-NAME and 4.25% Dianeal were examined. As expected, the initial Dianeal period produced

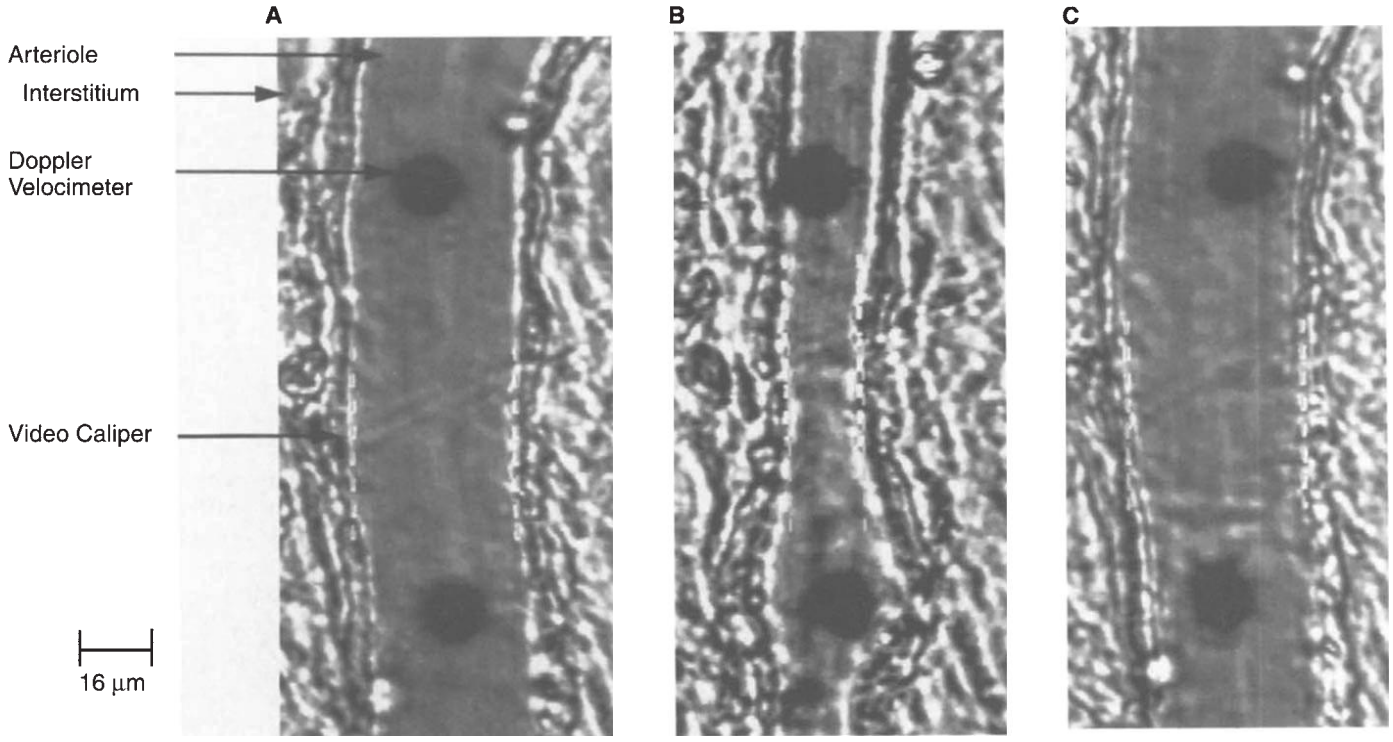



**Fig. 7.** *Photomicrograph of an intravital microscopic image of an arteriole during an experiment with ADMA.* **A.** The baseline diameter (33 μm). **B.** ADMA induced vasoconstriction (16 μm). **C.** The reversal of the ADMA induced vasoconstriction by 4.25% Dianeal (38 μm). The dotted white lines in A represent the video caliper, and the two black dots in the vessel represent the optically placed doppler velocimeter. Magnification 200×.

vasodilation when compared to the baseline period (32.6 ± 3.2 vs. 28.0 ± 2.5 μm, $P < 0.01$, $N = 5$). Despite the addition of 100 μm L-NAME to the 4.25% Dianeal, no vasoconstriction occurred in any time period during which the nitric oxide synthesis inhibitor was present. Furthermore, when compared to the baseline period, the vessel remained vasodilated for all time periods during which L-NAME was simultaneously superfused with the Dianeal ($P \leq 0.01$ and $P \leq 0.05$; Fig. 8; $N = 5$). Following the L-NAME plus Dianeal period, Dianeal was again superfused but no additional vasodilation occurred. There was no significant difference in arteriole diameter for the initial Dianeal period versus L-NAME plus Dianeal period versus the final Dianeal period. In regard to the $V_{RBC}$, there was no significant difference in the L-NAME plus Dianeal period versus the baseline period or the final Dianeal period. One point of significant difference did occur in the 70- to 80-minute superfusion time period (which corresponds to the last 10 min of the L-NAME in Dianeal period) as compared to the 10- to 20-minute time period (which corresponds to the last 10 min period of the initial Dianeal period; $P < 0.05$). Thus, 4.25% Dianeal completely prevented the L-NAME induced vasoconstriction and the L-NAME induced decrease in $V_{RBC}$ with the exception of one 10-minute time period. The transient arteriolar vasoconstrictive effects of peritoneal dialysis solutions previously described by Miller et al in small arterioles (baseline diameter approximately 8 to 10 μm, [11]) was an inconsistent finding in arterioles with a baseline diameter 20 to 35 μm (occurring in only 2 of 5 arterioles in Experimental Group 4). Finally, there were no significant differences in blood pressure in any Experimental Group when comparing the baseline period to two 30-minute inhibitor time periods and the Dianeal periods.

## Discussion

This study confirms previous observations that basal levels of nitric oxide are important in maintaining normal arteriolar vascular tone and blood flow in the mesenteric microcirculation [1, 2]. Both the exogenous inhibitor, L-NAME, and the endogenous inhibitor, ADMA, produce marked arteriolar vasoconstriction and diminished blood flow. L-NAME results in maximum vasoconstriction to 74% of baseline diameter (combining the results from the L-NAME induced vasoconstriction in Experimental Groups 1 and 3), and ADMA results in maximal vasoconstriction to 68% of baseline diameter. Both inhibitors decrease blood flow as shown by a decrement in $V_{RBC}$ to 44% of baseline for L-NAME and 52% of baseline $V_{RBC}$ for ADMA. These effects are time dependent with the maximal effects occurring in the last 20 minutes of the 60-minute inhibitor period. Since basal levels of nitric oxide production are critical in maintaining normal arteriolar microvascular hemodynamics, elevations of plasma levels of endogenous nitric oxide synthesis inhibitors, such as what occurs in renal failure, potentially could alter the microvascular hemodynamics during peritoneal dialysis.

Despite the marked arteriolar hemodynamic changes which occur with inhibition of nitric oxide production, standard peritoneal dialysis solutions such as 1.5% and 4.25% Dianeal reverse these microvascular effects. Both the 1.5% and 4.25% Dianeal solutions rapidly (usually within the first 5 min of exposure to Dianeal) reverse the vasoconstriction and diminished blood flow produced by nitric oxide synthesis inhibition. Both solutions restore the arteriole diameter and blood flow back to baseline values. Since these peritoneal dialysis solutions remain vasoactive

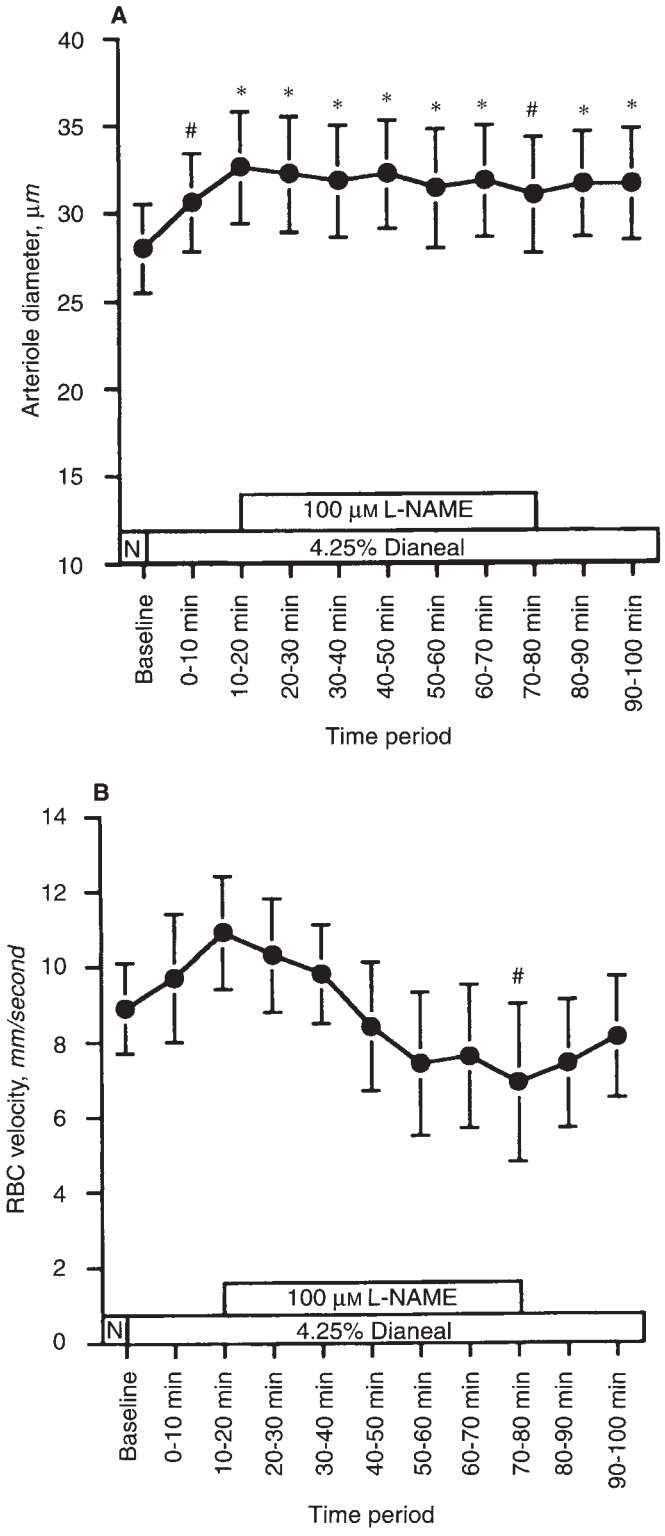



**Fig. 8.** *Hemodynamic data for Experimental Group 4.* **A.** Arteriole diameter. 4.25% Dianeal (initial period), 4.25% Dianeal plus L-NAME and 4.25% Dianeal (final period) all produced vasodilation when compared to the baseline period, $\#P \leq 0.05$ and $*P \leq 0.01$. **B.** Red blood cell velocity ($V_{RBC}$). No significant change in $V_{RBC}$ in any time period except in the final ten minutes of Dianeal plus L-NAME period (70 to 80 min) when compared to the last ten minute period of the initial Dianeal period (10 to 20 min), $P < 0.05$, $N = 5$.

despite nitric oxide synthesis inhibition, this suggests that these solutions possess vasoactive properties largely through a nitric oxide independent mechanism.

These observations, however, do not negate the possibility that hyperosmolar solutions produce nitric oxide dependent vasodilation. Previous studies of intestinal submucosal arterioles have demonstrated that increasing the osmolarity of the submucosal interstitial space with hypertonic saline or mannitol produced a dose-dependent arteriole vasodilation [14]. This hyperosmolar induced vasodilation was attenuated when endothelial derived relaxing factor (nitric oxide) synthesis was inhibited by L-NAME. Interestingly, the authors note that L-NAME attenuated 400 mOsm saline-induced vasodilation by approximately 50% but equilmolar L-NAME attenuated 400 mOsm mannitol-induced vasodilation by only 22%. These data suggest that the composition of the hyperosmolar solution is important in determining the amount of nitric oxide induced vasodilation. Furthermore, these hyperosmolar solutions still produced vasodilation over baseline values despite nitric oxide production inhibition. Therefore, for hyperosmolar solutions at least two vasodilatory mechanisms are in effect, one being nitric oxide independent and the other nitric oxide dependent. The degree of nitric oxide induced arteriole vasodilation appears to be related to the composition of the hyperosmolar solution.

To further investigate the possibility that peritoneal dialysis solutions could have a partial component of nitric oxide dependent vasodilation, a group of experiments (Experimental Group 4) were performed in which the arteriole was first exposed to the peritoneal dialysis solution followed by simultaneous superfusion of the nitric oxide synthesis inhibitor and the peritoneal dialysis solution. If these peritoneal dialysis solutions produce significant vasodilation partially through a nitric oxide dependent mechanism, then there should occur some relative vasoconstriction when comparing the initial Dianeal period with the L-NAME plus Dianeal period. The initial exposure of Dianeal produced the expected vasodilation over baseline diameters. However, when the Dianeal and L-NAME were simultaneously superfused, the arteriole remained significantly vasodilated above the baseline diameter throughout the entire L-NAME plus Dianeal period. In addition, there was no significant difference in arteriole diameter between the initial Dianeal period and the L-NAME plus Dianeal period. Thus Dianeal appears to be vasoactive primarily through a nitric oxide independent mechanism.

ADMA, methylguanidine and aminoguanidine are endogenous guanidino compounds which accumulate in renal failure and inhibit nitric oxide production [18]. ADMA appears to be the most potent endogenous inhibitor, with methylguanidine a weak inhibitor, and aminoguanidine inhibiting only the inducible form of nitric oxide synthase. ADMA is reported to accumulate in renal failure to plasma concentrations of approximately 5 μM [8, 9]. In addition, it has also been suggested that local concentrations of ADMA may be greater than the observed plasma concentrations occurring in renal failure [2, 17–18]. In this investigation the experimental concentrations of the nitric oxide synthesis inhibitors ADMA and L-NAME are approximately twenty times greater than the increased plasma concentrations of ADMA present in ESRD patients. Therefore, if the peritoneal dialysis solutions attenuate and reverse the arteriolar hemodynamic effects of ADMA at the present experimental concentrations (100

$\mu$M), then these dialysis solutions would be expected to overcome any mesenteric arteriolar hemodynamic effects of ADMA observed in ESRD.

Finally, it is well established that nitric oxide inhibition produces an inflammatory reaction in postcapillary venules, as evidenced by increased leukocyte adhesion to the venular endothelium and leukocyte migration into the interstitial tissue [4]. In this investigation no increases in leukocyte endothelial adhesive interactions were observed in the arterioles with nitric oxide inhibition. This is consistent with previous observations that the inflammatory reaction in the mesenteric microcirculation, as evidenced by alterations in leukocyte kinetics, is primarily confined to the postcapillary venule [4, 19–20].

In summary, this study confirms that basal levels of nitric oxide are important in maintaining normal vascular tone and blood flow in the mesenteric microcirculation. Superfusion of the nitric oxide synthesis inhibitors ADMA and L-NAME produces significant vasoconstriction and diminishes blood flow in mesenteric arterioles. The arteriolar hemodynamic effects of nitric oxide synthesis inhibitors are reversed by a standard hypertonic peritoneal dialysis solution (Dianeal). The vasoconstrictive effects of L-NAME are completely prevented when arterioles are simultaneously superfused with 4.25% Dianeal and L-NAME. This suggests that this hypertonic peritoneal dialysis solution produces vasodilation primarily independent of nitric oxide mediated mechanisms. Finally, reversal of the arteriolar hemodynamic effects of nitric oxide inhibition by Dianeal suggests that the endogenous inhibitor of nitric oxide production, ADMA, has no significant effects in the regulation of the mesenteric microvascular arteriolar hemodynamics during peritoneal dialysis.

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